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ENERGETICS OF PERMEATION OF THIN LIPID MEMBRANES BY IONS

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SUMMARY

The energy of an ion in a thin hydrocarbon membrane relative to its energy in a bulk aqueous phase is considered in terms of the electrostatic and surface components that may be expected to be involved. Except when diffusion activation energies are large compared to partition free energies, the latter will control permeation rate and the state of an ion having the lowest partition energy will be critical for its permeability. This minimum is found when an ion is surrounded with a thin layer of water. All ions of the same charge will tend to be at their lowest state in a sphere of water of the same size. It is concluded, therefore, that all ions of a given charge will have about the same permeability in lipid membranes.

INTRODUCTION

A characteristic of bimolecular lipid membranes, both of the planar film and vesicle types, is an extremely low permeability to most inorganic ions. This low permeability is generally attributed to the large energies involved in transferring a small ion from an aqueous phase of high dielectric constant to a lipid membrane of low dielectric constant. Although such energies make an important contribution to the barrier properties of lipid bilayers, there are reasons for believing that other physical phenomena must also be involved. Since dielectric polarization energies depend upon the field strength at the surface of an ion, for isoionic series, the energy of transfer from high to low dielectric constant media must increase with decreasing ionic size. In contrast to this prediction, planar lipid bilayers of uncharged lipids exhibit small diffusion potentials in the presence of salt gradients, negligible biionic potentials, and similar conductances in solutions of different electrolytes [1]. Similarly, phosphatidylcholine liposomes exhibit similar permeabilities to all of the alkali cations that have been tested [2, 3]. These vesicles do exhibit different isotope exchange rates for alkali cations and halide anions [2], but this is evidently due to the presence of a carrier which forms an uncharged complex with the anion [4]. Although it may be that the lack of apparent selectivity seen in measurements of electrical properties of planar bilayers is due to ionic impurities which carry more current than the ions forming the majority in the aqueous phases, such impurities cannot account for the lack of discrimination among alkali cations exhibited by uncharged lipid vesicles. The purpose of this communication is to suggest that if interfacial energies involved in permeation are taken into account, selectivity among small ions should indeed be small.

RESULTS AND DISCUSSION

One of the earliest analyses of the role of dielectric polarization energies in ion transport across lipid layers was given by Parsegian [5]. The following draws heavily from that work. The energy of interaction of a spherical charge with a dielectric medium (the "self energy") (E'_e) depends upon the dielectric constant of the medium (D_m), the ionic charge (e), and the radius of the charge (r_i) according to

$$E'_e = \frac{e^2}{2\,D_m r_i} \tag{1}$$

The reversible work necessary to transfer an ion from water ($D = 80$) to the interior of a hydrocarbon phase ($D = 2$) is simply the difference between two terms given by Eqn. 1 containing the appropriate values of the dielectric constant. In the case of a membrane containing a charge having a radius that is not negligible with respect to the thickness of the membrane, the transfer energy is not as large as it would be if the charge were far removed from the water surface. For a lipid bilayer with a hydrocarbon region of thickness of 40 Å, the energy of an ion will exceed that in the aqueous phase by [5]

$$\Delta E = \frac{e^2}{2r_i}\left(\frac{1}{D_o} - \frac{1}{D_w}\right) - \frac{e^2}{D_o t}\ln\left(\frac{2D_w}{D_w + D_o}\right) \tag{2}$$

where t is the thickness of the membrane and subscripts w and o refer to aqueous and lipid phases, respectively.

Eqn. 2 has been plotted in Fig. 1 as the curve marked ΔE_e. Since the crystal radii of the alkali cations vary between about 0.7 Å for Li^+ to about 1.5 Å for Cs^+, the ions of this series would reside in a thin lipid membrane at very much different energies (see inset, Fig. 1). The transfer energy for all alkali ions is predicted to be much larger than the activation energy for diffusion (even if the membrane had a viscosity of 1000 centipoise, the activation energy for diffusion would still be less than 10 kcal/mol), so the transfer energy should be roughly equal to the activation free energy for permeation of the membrane by an unhydrated ion. On the basis of absolute rate theory [6], the permeabilities of the alkali cations (and anions, as well) should vary over a range in excess of 10^{10}. Even the activation energies of neighboring ions such as Na^+ and K^+ should differ by more than 20 kcal, a difference sufficiently large that the permeability of Na^+ should be negligible in comparison with that of K^+. This prediction stands in contrast to the observation of equal permeability of lecithin liposomes [3].

Activation energies and discrimination among ions of different sizes would obviously be lower if it were not the unhydrated but rather the hydrated ion that permeates the membrane. Hydration would reduce the difference between the smallest and largest ions of a series because the ions with the smallest crystal radius are most strongly hydrated, however, it would tend to overcorrect; on the basis of hy-

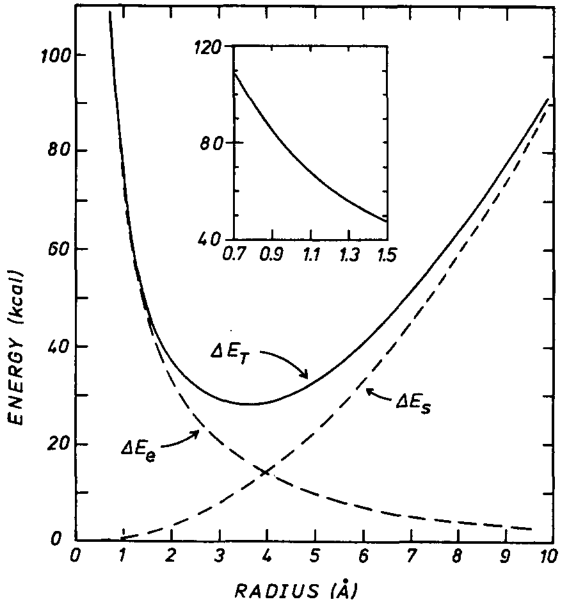

Fig. 1. Free energy of transfer of a sphere bearing one electronic charge from bulk water to a 40 Å thick lipid membrane as a function of the radius of the sphere. ΔE_e, dielectric polarization energy component. ΔE_s, interfacial energy component. E_T, sum of ΔE_e and ΔE_s. D_w taken as 80, D_o taken as 2, and $\gamma_{o/w}$ taken as 50 dyne/cm. Inset, plot, on expanded abscissa, of ΔE_e curve from 0.7 Å to 1.5 Å.

drated radii, K^+ would be expected to have an activation free energy of about 10 kcal more than that of Na^+. This would lead to a prediction by absolute rate theory that the permeability of the latter should exceed that of the former by more than a million times.

At this point it becomes appropriate to ask whether an ion at its lowest free energy in a lipid phase would necessarily be associated with the same amount of water that is normally considered to be the water of hydration in bulk water. Obviously, the more water an ion could bring into the lipid phase, the lower would be the field strength at the surface of such an aqueous sphere, and the lower would be the electrostatic transfer energy. Carried to its logical extreme, this argument would suggest that the lowest energy state for an ion in a lipid phase would be if it were surrounded by a sphere of water of infinite radius. Clearly this would not occur, but it is revealing to inquire why not. Let us consider the process of taking from bulk water to bulk lipid, an ion surrounded by a rather thick layer of water. This process simply amounts to creating an oil-water interface with an area equal to that of the sphere surrounding the ion. (This conclusion may be more obvious if the process is analyzed as the sum of hypothetical steps: 1. A sphere of area A is removed from the aqueous phase into air. The energy invested in the system would be $A\gamma_{a/w}$. 2. A spherical pocket of air of area A is created in the lipid phase. This will require an energy investment of $A\gamma_{a/o}$. 3. The water sphere is placed in the pocket in the lipid. In this process the air-water and air-oil interfaces are destroyed and an oil-water interface is created. The energy investment is $-(A\gamma_{a/w}+A\gamma_{a/o}-A\gamma_{o/w})$. 4. The total surface energy for the transfer process is the sum of the energies of the individual steps, or, $A\gamma_{o/w}$. For a sphere of radius r this is given by

$$\Delta E_s = 4\pi r^2 \gamma_{o/w} \tag{3}$$

where ΔE_s represents the component of the transfer energy depending upon surface forces and $\gamma_{o/w}$ is the interfacial tension between water and the hydrocarbon interior of the lipid bilayer. This energy increases rapidly with the radius of the sphere and is evidently what prevents an ion from assuming its lowest electrostatic free energy in the interior of a medium of low dielectric constant.

Given that the surface energy part of the transfer energy increases with increasing radius of a water-covered ion and that the electrostatic part decreases with increasing radius thereof, the situation in which an ion in a hydrocarbon is in the lowest free energy state is when it is surrounded by an amount of water such that the sum of ΔE_e and ΔE_s is at a minimum. There are a number of difficulties in accurately evaluating either of the relevant energies, but they can both be approximated by using macroscopic parameters and assuming that molecular interfaces are smooth. Despite the crudeness of the model, it will be seen that some qualitative conclusions can be drawn from it.

To calculate the dependence of ΔE_s on the radius of the water sphere within which an ion is assumed to be transferred, the simplifying assumption will be made that the interfacial energy between lipid hydrocarbon and the surface of the wet ion is equal to that between water and hydrocarbons such as hexadecane (50 ergs/cm^2), and that it is independent of the radius of the water sphere. Given such an assumption, the curve marked ΔE_s in Fig. 1 is obtained from Eqn. 3. Surface energies can be appreciable for aqueous spheres of radii greater than a few Å.

The electrostatic energy of an ion of radius r_i within a sphere of water of radius r which is immersed in a medium of dielectric constant D_m is given by [5]

$$E_e = \frac{e^2}{2D_m r} + \frac{e^2}{2D_w}\left(\frac{1}{r_i} - \frac{1}{r}\right) \tag{4}$$

The free energy of transfer of this sphere from water ($D = 80$) to lipid ($D = 2$) becomes simply,

$$\Delta E_e = \frac{e^2}{2r}\left(\frac{1}{2} - \frac{1}{80}\right) \tag{5}$$

When the influence of the thickness of the membrane is taken into account, Eqn. 5 becomes identical to Eqn. 2 and consequently corresponds to the curve in Fig. 1 marked ΔE_e. Eqn. 5 is, however, somewhat more of an approximation than is Eqn. 2 because it does not take electrostriction in the primary hydration shell into account. According to the arguments given above, the sum of the two transfer energy components should give the total energy of transfer as a function of the radius of the wet ion. This sum is given as ΔE_T in Fig. 1. According to this approximate treatment, all ions having crystal radii smaller than the radius at the minimum of E_T should permeate membranes with the same activation free energy and hence have essentially the same permeabilities. This result comes about because the relevant equations do not contain the radius of the ion, only the radius of the water sphere, which, according to Fig. 1, should be slightly under 4 Å. Ions with radii larger than 4 Å would not be constrained to follow the ΔE_s curve and would simply permeate as bare ions, the energy of transfer being lower, the larger the radius. It should be noted that with increasing charge on an ion, ΔE_e of Fig. 1 is increased. The minimum energy would therefore be higher and at a larger r, the greater the charge. Permeabilities should therefore decrease with increasing charge (of either sign).

This treatment, crude though it is, reveals that surface energies are considerable and suggest that, to a large extent, they compensate for differences of dielectric polarization energies between small ions of different crystal radius. The result is that most small ions seek a residence state in a lipid film such that they are all at a minimum energy state in a small water sphere of about the same size. Although more exact calculations may yield different curves than those of Fig. 1 and may even reveal that the dependence of ΔE_e and ΔE_s will vary somewhat with ionic radii, this would not alter the basic conclusion, namely, that partition of an ion into a hydrocarbon phase depends upon a balance of forces such that the effects of different crystal radii tend to be minimized.

The energy at the minimum of ΔE_T (Fig. 1) is about 28 kcal/mol. This is probably sufficiently larger than activation energies for interfacial transfer or any diffusion steps within the membrane so that it may be expected to be rate limiting. There are no experimental values of activation free energies that we are aware of, although there are several measurements of activation enthalpies. It is noteworthy that to the extent that the present treatment is correct, it is likely that experimental measurements based on temperature variation would yield incorrect results. Such measurements assume the barrier height to be temperature independent, whereas both dielectric constants and interfacial tensions have substantial temperature coefficients.

Since both dielectric constant and interfacial tension decrease with increasing unsaturation of a hydrocarbon, the permeability of bilayers should, on the basis of the model, increase with the increasing unsaturation of the constituent fatty acids.

Finally, it may be suggested that the increasing permeability of lipid bilayers in the presence of chloroform [7] or alcohol narcotics [8] is explicable on the basis of a model similar to that presented above. Depending upon their equilibrium concentration and polarizability (or polarity) such substances could stabilize an ion in a lipid phase to a greater degree than water molecules. Molecules with dielectric constants greater than that of the lipid would actually be electrostatically attracted (even though they are uncharged) to a permeating ion and could conceivably replace part of the water shell before an ion had penetrated very far into the bilayer (and hence before the barrier was at its maximum), and further reduce both interfacial and electrostatic free energies of the ion. Presumably, any somewhat polar molecule with a higher solubility in lipid than a "wet" ion would tend to increase the permeability of such ions. These molecules might be considered to be "dissociated ionophores" although they would be poorly selective and less efficient than true ionophores because of the entropy decrease necessary for their assembly around an ion.

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